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Precipitation affects soil microbial and extracellular enzymatic responses to warming

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ABSTRACT

Soil microbial communities and extracellular enzymes have important roles in many terrestrial ecosystem processes and are influenced by climate change drivers. In the present study, the individual and/or interactive effects of climate change drivers on soil microbial communities and extracellular enzyme activities were measured in experimental field plots planted with *Pinus densiflora* and subjected to air warming (ambient or +3 °C) in combination with precipitation treatments (reduced by 30%, ambient or elevated by 30%). Soil microbial biomass and four extracellular enzyme activities were measured. Additionally, the structure and composition of microbial communities were assessed. Warming increased microbial biomass nitrogen concentration by 22.3% in precipitation control plots and by 17.9% in elevated precipitation plots. Warming lowered the extracellular enzyme activity in precipitation control plots, but increased their activity in elevated precipitation plots. Warming had a differential effect on the composition of bacterial and fungal communities under different precipitation treatments, with significant changes in therelative abundance of Proteobacteria and Acidobacteria. Meanwhile, the alpha diversity index of both bacterial and fungal communities were affected by warming, with variation among the precipitation treatments. Changes in enzyme activities and microbial communities were correlated with shifts in soil environmental conditions (e.g., moisture, temperature, and available nutrients). In conclusion, changes in soil environmental conditions may select for distinct soil microbial communities, further reshaping soil ecosystem processes and functions in a warmer world.

1. Introduction

Ongoing global climate change is predicted to alter the soil microbial communities through direct and indirect pathways (Brockett et al., 2012). Changes in soil microbial assemblages may alter terrestrial ecosystem processes, including the decomposition of soil organic matter (SOM), carbon (C) sequestration, and nutrient cycling (Allison and Treseder, 2008; Schindlbacher et al., 2011). Direct effects of climate change are the result of altered microbial metabolism and substrate availability (Classen et al., 2015), while indirect effects arise from soil microbial-plant interactions that reflect the change in growth, phenology, and species composition of plants due to climate change (Brockett et al., 2012; Classen et al., 2015).

Temperature and precipitation are the most important climate change drivers that alter the structure and composition of soil microbial communities in terrestrial ecosystems. Increases in temperature can enhance the activity, biomass, alpha diversity, and respiration of soil microbes by accelerating metabolic rates, thereby favoring microbial communities adapted to warmer environments (Zhang et al., 2005; Bell et al., 2010; Chen et al., 2015). Meanwhile, previous studies suggested that drought may reduce the microbial activity and population size by reducing soil moisture (Schimel et al., 2007; Sheik et al., 2011; Manzoni et al., 2012), while elevated precipitation may enhance the microbial respiration by increasing soil available substrates (Xiang et al., 2008). Alteration of the precipitation pattern may also affect the soil microbial community by increasing the ratio of fungi to bacteria by as much as 50% due to the changes in C inputs to soil from plants, altering the microbial community composition (Castro et al., 2010; Zhao et al., 2016).

Extracellular enzymes are useful indicators of soil fertility as they are major drivers of the decomposition of soil organic matter: β-1,4-glucosidase (BG) is involved in C acquisition by catalyzing cellulose degradation; *N*-acetyl-glucosaminidase (NAG) and L-leucine aminopeptidase (LAP) are involved in the mineralization of nitrogen (N) from chitin and proteins, as well as the breakdown of fungal cell walls; acid phosphatase (AP) is involved in the mineralization of phosphorus (P; Zhou et al., 2013). Though both microbes and plant roots may secrete extracellular enzymes, the activity of extracellular enzymes is often

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representative of microbial functions (Puissant et al., 2015; Souza et al., 2017). Higher soil temperature could either increase or decrease the soil extracellular enzyme activities. It can not only stimulate enzyme production, resulting in greater potential for soil extracellular enzyme activity (Henry, 2013), but also reduce the rate of enzyme-substrate binding, decelerating enzyme-mediated reactions. These contradictory effects may occur because not all enzymes respond consistently to increases in temperature. Higher soil temperature can increase the activities of NAG and AP by enhancing the soil microbial activity (Brzostek and Finzi, 2011; Zhou et al., 2013), whereas it can decrease the activities of BG, NAG, and LAP by reducing soil moisture through elevated evapotranspiration (Allison and Treseder, 2008; Zhou et al., 2013; Machmuller et al., 2016). Similarly, soil extracellular enzyme activities do not respond consistently to altered precipitation. Sardans and Peñuelas (2005) showed that drought significantly decreased the activity of BG by 47%, but did not induce significant shifts in AP activity. Conversely, Kreyling et al. (2008) found that elevated precipitation significantly increased NAG activity, while neither elevated precipitation nor drought induced significant changes in BG and AP activities. Zhou et al. (2013) also reported that 30% elevated precipitation significantly decreased BG activity, but increased NAG, LAP, and AP activities.

In many parts of the world, concurrent changes in temperature and precipitation patterns are occurring due to climate change, leading to interactive effects of temperature and precipitation on soil properties, microbial communities, and extracellular enzyme activities (Zhang et al., 2016a). However, it is challenging to predict the outcome of emerging temperature and precipitation regimes. For instance, soil warming of 3.0 °C increased the soil temperature by as much as 2.3 °C and also stimulated evapotranspiration, which made the soil drier despite a concurrent 30% increase of precipitation (Li et al., 2017a). Meanwhile, higher precipitation (30% more than the ambient conditions) also had a cooling effect on soil temperature, reducing it by 0.7 °C compared to soils with warming alone (Li et al., 2017a). Lower precipitation (50% less than the ambient conditions) is expected to limit water availability and reduce the diffusion of substrates, thereby constraining microbial biomass and enzyme (BG, NAG, and LAP) activities, resulting in similar microbial and enzymatic responses in soil warmed by 4 °C compared to soils with no water limitation (Steinweg et al., 2013). Since soil biological responses are dependent upon the interaction between temperature and precipitation, it is essential to evaluate warming effects on soil microbes and extracellular enzymes while considering a precipitation gradient that includes drier and wetter regimes (Brockett et al., 2012; Henry, 2013; Zhang et al., 2016a).

To investigate how warmer air temperature and precipitation manipulation affect the soil microbial communities and extracellular enzyme activities, we set out to sample a constructed open-field experimental site planted with *Pinus densiflora* seedlings, which was established two years prior to this study. Previous results from this experimental site experimental site demonstrated that warming increased soil respiration, but the magnitude of warming-induced respiration varied among precipitation (Li et al., 2017b). The specific goals of the present study were (1) to quantify the main and interactive effects of warming and precipitation regimes on soil microbial biomass, composition, and structure, and (2) to evaluate the main and interactive effects of warming and precipitation regimes on four extracellular enzymes, namely the hydrolytic enzymes BG, NAG, LAP, and AP involved in organic C, N and P cycling. The study was predicated upon four hypotheses: (1) warming will increase soil microbial biomass and extracellular enzyme activity, (2) elevated precipitation will increase soil microbial biomass and extracellular enzyme activity, whereas lower precipitation will reduce soil microbial biomass and extracellular enzyme activity, (3) due to the interaction of warming and precipitation, the greatest soil microbial biomass and extracellular enzyme activity will occur in the warmest, wettest soil conditions, and the lowest soil microbial biomass and extracellular enzyme activity will occur in the coolest, dry soil conditions, and (4) the diversity and structure of different soil microbial communities (i.e., bacterial and fungal communities) will respond differently to warming and precipitation manipulation.

2. Materials and methods

2.1. Experimental site description and design

The experiment was conducted at a nursery located at Korea University, Seoul, South Korea (37°35′36″ N, 127°1′31″ E). The nursery was used to study the effect of warming on the representative tree species in South Korea from 2010 to 2013. The interactive effects of warming and precipitation manipulation were evaluated in the nursery from 2013 to 2015. At this site, the long-term (1981–2010) annual air temperature was 12.5 °C and annual natural precipitation was 1450.5 mm, which is cooler and wetter than the mean air temperature of 13.6 °C and annual precipitation of 792.1 mm recorded in Seoul in 2015 (Tables S1 and S2; Korea Meteorological Administration, 2017). Before the site for this study was established in April 2013, the soil was plowed to a depth of 50 cm and all vegetation was removed. The experiment consisted of 18 plots (1.5 m × 1.5 m with a 50 cm buffer between the plots) that were planted with 45 seedlings (at a spacing of 15 cm between the seedlings) of 2-year-old *P. densiflora* with a similar size (i.e., 22.1–24.0 cm height and 5.3–5.8 mm root collar diameter) in April 2013. *P. densiflora* is a common coniferous tree in temperate regions and a dominant, economically important species in mixed and natural coniferous forests of South Korea, covering approximately 23.1% (1,477,000 ha) of the total forest area and 55% of the coniferous forest area in South Korea (Noh et al., 2016; Lee et al., 2017). The soil is a loamy sand (80% sand, 14% clay, and 6% silt) with slightly acidic pH (6.5 ± 0.0; mean ± standard error), low cation exchange capacity (CEC; 6.1 ± 0.1 $cmol_c\ kg^{-1}$) and total C and N concentrations of 1.9 ± 0.0 $g\ kg^{-1}$ and 0.1 ± 0.0 $g\ kg^{-1}$ with C:N ratio of 16.0 ± 0.2.

The field plots were monitored from April 2013 to December 2015. The experimental design was a complete factorial with six treatments and three replicates (18 plots in total), consisting of two levels of air warming (control (C) and +3 °C based on air temperature (W)) in combination with three levels of precipitation manipulation (−30% (P-), control (P0), and +30% (P+)). Factorial treatments with ambient air temperature are referred to as C*P-, C*P0 and C*P+, and those with air warming were designated as W*P-, W*P0 and W*P+, which reflects the combined effects of warming and precipitation regimes. Air warming and precipitation manipulation treatments were selected based on the climate change scenario RCP 8.5 for South Korea, which simulates climate change for the next 50 years (Kim et al., 2014). The air warming treatment was achieved with an infrared lamp heater consuming 1000 W of electric power (FTE-1000; Mor Electric Heating Instrument Inc., Grand Rapids City, Michigan, USA), which was set at a height of 60 cm above the *P. densiflora* seedling canopy to increase air temperature by 3 °C. A controller consisting of a data logger and a relay automatically turned on the heater if the difference in air temperature between the unwarmed and warmed plots was lower than 3 °C. Nonetheless, the actual difference in air temperature (2.8 ± 0.1 °C; verified with an infrared temperature sensor, see subsection 2.3) was slightly lower than 3 °C due to time lag between data logging and heating as well as seasonally intensive rainfall events that cause cooling at the site (Yun et al., 2014).

Precipitation was manipulated with polycarbonate panels, an automatic pump, and a drip-irrigation system. The transparent polycarbonate panels were installed above the reduced precipitation plots and covered 30% of the plot area to block precipitation, reducing annual precipitation to the reduced precipitation plots by 30%. The blocked precipitation was immediately diverted to the elevated precipitation plots by an automatic pump and a drip-irrigation system, which added 30% more precipitation to the elevated precipitation

plots. A polycarbonate panel with gaps to permit rainfall infiltration was installed in the control and elevated precipitation plots to avoid any effects on *P. densiflora* seedlings due to shading or heat trapping under the panel. The polycarbonate panels were permanently above the plots throughout the year, except for 2 months in winter because snow accumulation could damage the panels.

2.2. Soil collection and sample preparation

Composite soil samples were acquired by mixing soil samples (0–15 cm) collected from ten random points in each plot with a soil corer (2.54 cm in diameter) once every two months from April 2015 to December 2015 (a total of five times). After sieving (< 2 mm) and removing visible plant material, soil samples were kept in a refrigerator at 4 °C until they were analyzed for soil physicochemical properties, microbial biomass, and extracellular enzyme activity. In addition, a portion of the sieved soil collected in August 2015 was stored at −20 °C for microbial community structure and composition analyses. Soil physicochemical and microbial analyses assays were performed within 2 weeks of soil sample collection, while extracellular enzyme activity was assayed within 48 h.

2.3. Assays for measurement of soil physicochemical properties

In each plot, air temperature, soil temperature at a depth of 5 cm, and soil moisture at a depth of 10 cm were recorded every 30 min using an infrared temperature sensor (SI-111), a temperature sensor (107-L34), and a reflectometer probe (CS616), respectively, connected to a CR3000 data logger (all sensors, probes and the data logger were from Campbell Scientific, Logan, Utah, USA). Soil pH was measured using a 1:5 air-dried soil to distilled water ratio. Soil CEC was measured by using the ammonium acetate (pH 7.0) method (Brown, 1943). Soil NH_4-N concentration was measured by using the salicylate method (Mulvaney, 1996), and the NO_3-N concentration was measured by using the Griess reagent and the VCl_3 reducing agent (Miranda et al., 2001; Doane and Horwáth, 2003). Total soil C (TC) and N (TN) concentrations were measured by dry combustion using an elemental analyzer (vario Macro, Elementar Analysensysteme GmbH, Germany).

2.4. Soil microbial biomass assay

Soil microbial biomass C (MBC) and N (MBN) concentrations were measured with the chloroform fumigation-direct extraction method (Brookes et al., 1985; Vance et al., 1987) after saturating the soil with water to minimize errors associated with the variable soil moisture content between plots (Witt et al., 2000). Soluble organic C and N concentrations in 0.5 mol L^{-1} K_2SO_4 extracts (1:4 soil:solution) of soil, extracted before and after 24 h of chloroform fumigation were analyzed with a total organic C analyzer equipped with a total nitrogen module (Shimadzu TOC-V CPH, Shimadzu Co., Kyoto, Japan). The difference in extractable organic C and N concentrations of non-fumigated and fumigated soils was assumed to be the C and N released from soil microbial biomass. The MBC and MBN concentrations were the soluble organic C and N concentrations released from microbial biomass, divided by the efficiency factor of 0.45 (Brookes et al., 1985; Fu et al., 2012).

2.5. Soil extracellular enzyme activity assay

Four extracellular enzymes involved in the degradation of organic C, N, and P compounds, namely, BG, NAG, LAP, and AP (Sinsabaugh et al., 2009; Table S3), were selected for this study. Enzyme activities were measured by using a fluorometric method (DeForest, 2009) using black polystyrene 96-well microplates (300 µl, SPL Life Sciences Co. Ltd, Pocheon-si, South Korea), with substrate analogs linked to fluorescent molecules, 4-methylumbelliferone and 7-amino-4-methylcoumarin (Sigma-Aldrich CO. Ltd, Yongin-si, South Korea). Citrate-phosphate buffer solution (pH 6.8) was used for enzymes BG, NAG, and AP, whereas Trizma buffer solution (pH 7.2) was used for the LAP enzyme, which buffered the enzyme-substrate solutions in a similar pH range as measured in soil from the experimental field plots (A'Bear et al., 2014).

Enzyme activity assays and calculations were based on the method of DeForest (2009). The samples, references, and substrates followed a strict order and position within microplate wells prior to placing microplates in a 25 °C incubator for 2 h (BG, NAG, and AP assays) or 4.5 h (LAP assay). To terminate the reaction, 50 µl of 0.2 mol L^{-1} NaOH solution was added to each well. Fluorescence was measured at 355-nm excitation and 460-nm emission wavelengths using a Multi-Detection Microplate Reader (Sense, HIDEX, Turku, Finland). Soil extracellular enzyme activities were expressed in nmol substrate cleaved g^{-1} dry soil h^{-1}.

2.6. Soil microbial community structure and composition assay

Soil microbial genomic DNA was extracted from each sample using a Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's protocol. The primer pair 343F (5′-TACGGRAGGCAGCAG-3′) and 798R (5′-AGGGTATCTAATCCT-3′) was used to amplify the V3-V4 region of bacterial 16S rDNA. The primer pair ITS1-F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) was used to amplify the fungal ITS-1 region. Polymerase chain reaction was conducted as follows: 95 °C for 2 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. Amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) following the manufacturer's protocols. Purified amplicons were quantified using QuantiFluor™ -ST (Promega, USA). They were then pooled in an equimolar ratio and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) following the standard protocols. Paired-end sequence reads were preprocessed using a Trimmomatic software (Bolger et al., 2014) to detect and remove ambiguous bases and low-quality sequences with average quality scores below 20. After trimming, paired-end reads were assembled using the FLASH software (Reyon et al., 2012). Parameters of assembly were: 20% maximum mismatch rate, 200 base pairs (bp) of maximum overlapping, and 10 bp of minimal overlapping. Sequence alignments underwent further noise reduction as follows: reads with ambiguous, homologous sequences fewer than 200 bp were discarded. Chimera sequences were removed using a QIIME v. 1.8.0 (Caporaso et al., 2010). The QIIME package was then used to apply a 97% similarity cutoff and cluster the sequences into operational taxonomic units (OTUs). All representative reads were annotated and BLAST searched against the Greengenes and UNITE databases using RDP classifier with a 70% confidence threshold.

2.7. Statistical analyses

Alpha diversity index (i.e., Chao 1, Shannon, Observed species, and Phylogenetic distance indexes) analyses were performed using the summary single command of the MOTHUR software. Repeated measure analysis of variance (ANOVA) was used to test the main effects of warming, precipitation manipulation, and time, as well as their interaction on soil physicochemical properties, microbial biomass, and extracellular enzyme activities at five sampling dates during the study period. Two-way ANOVA was used to test the main and interactive effects of warming and precipitation manipulation on alpha diversity indices of bacterial and fungal communities that were assessed in August 2015. When main or interactive effects were significant ($P < 0.05$), a post-hoc Tukey's honest significance test was used for mean comparisons between treatments. Redundancy analysis (RDA) was used to visualize the effect of treatments on extracellular enzyme

Table 1
ANOVA results and mean soil physicochemical properties for each treatment during the study period, presented as mean ± standard error.

	Treatments						Main effects and interactions
	C*P-	W*P-	C*P0	W*P0	C*P+	W*P+	
Air temperature (°C)	15.8 ± 4.3	18.2 ± 4.3	15.6 ± 4.2	18.5 ± 4.4	15.5 ± 4.2	18.3 ± 4.4	W**; T**
Soil temperature (°C)	17.1 ± 4.0	17.4 ± 4.0	16.5 ± 4.0	17.5 ± 4.0	16.8 ± 4.0	17.3 ± 4.0	W*; T**; T × P*; T × W**
Soil moisture (Vol %)	6.7 ± 0.3ab	5.7 ± 0.4b	7.4 ± 0.4a	5.8 ± 0.3b	7.4 ± 0.4a	6.4 ± 0.3ab	W**; T**
pH	6.6 ± 0.1	6.6 ± 0.1	6.6 ± 0.0	6.6 ± 0.0	6.4 ± 0.0	6.4 ± 0.1	P*; T**
CEC ($cmol_c$ kg^{-1})	6.1 ± 0.2	6.3 ± 0.2	6.1 ± 0.3	6.2 ± 0.1	5.9 ± 0.2	5.9 ± 0.2	T**; T × P × W*
TC (g C kg^{-1} Soil)	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	T*; T × W*
TN (g N kg^{-1} Soil)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	T**; T × P**; T × W*; T × P × W*
TC/N	15.5 ± 0.2	15.2 ± 0.4	16.8 ± 0.3	17.2 ± 0.6	16.0 ± 0.4	15.4 ± 0.2	P*
NH_4-N (mg NH_4-N kg^{-1} Soil)	5.5 ± 0.8	4.9 ± 0.6	5.7 ± 0.7	5.5 ± 0.7	5.6 ± 0.8	5.4 ± 0.5	T**
NO_3-N (mg NO_3-N kg^{-1} Soil)	1.2 ± 0.3	1.1 ± 0.4	1.4 ± 0.3	1.1 ± 0.2	1.0 ± 0.2	1.3 ± 0.4	T**
IN (mg N kg^{-1} Soil)	6.7 ± 1.0	6.0 ± 0.9	7.1 ± 0.8	6.6 ± 0.9	6.6 ± 0.8	6.8 ± 0.7	T**

W = warming; P = precipitation manipulation; T = time; C*P- = air temperature control and reduced precipitation; W*P- = air warming and reduced precipitation; C*P0 = air temperature control and precipitation control; W*P0 = air warming and precipitation control; C*P+ = air temperature control and elevated precipitation; and W*P+ = air warming and elevated precipitation. CEC = cation exchange capacity; TC = total carbon; TN = total nitrogen; TC/N = ratio of TC to TN; and IN = inorganic nitrogen. Different lower-case letters represent significant differences ($P < 0.05$). ns = not significant at the level of $P > 0.05$; * = significant at the level of $P < 0.05$; and ** = significant at the level of $P < 0.01$.

activities during the study period, and the relative differences in compositional variations in bacterial and fungal communities among treatments. RDA was performed using CANOCO v.4.5 (Ithaca, NY, USA). Other statistical analyses were done using SAS v.9.3 (SAS Systems, Cary, NC, USA).

3. Results

3.1. Soil physicochemical properties

During the study period, air temperature, soil temperature, and soil moisture were significantly affected by warming ($P < 0.001$, $P = 0.013$, and $P = 0.001$, respectively; Table 1). Warming increased air temperature by 2.4 °C, 2.9 °C, and 2.8 °C on average in the reduced, control, and elevated precipitation plots, respectively. Warming induced less change in soil temperature than air temperature, and the soil temperatures increased by an average of 0.3 °C, 1.0 °C, and 0.5 °C in the reduced, control, and elevated precipitation plots, respectively (Table 1). Warming affected soil moisture significantly and created drier conditions in the W*P- and W*P0 plots than the C*P0 and C*P+ plots ($P = 0.007$). Specifically, plots with the warming treatment had lower soil moisture, on average 1.0 Vol%, 1.6 Vol%, and 1.0 Vol% less moisture in the reduced, control, and elevated precipitation plots, respectively (Table 1 and Fig. 1). Precipitation manipulation resulted in a significant ($P = 0.040$) decline in soil pH and also affected the TC:TN ratio significantly ($P = 0.029$). Reduced precipitation in the unwarmed plots lowered the TC:TN ratio by 8.0%, on average, while elevated precipitation resulted in a decline of 4.9% in the TC:TN ratio (Table 1).

3.2. Soil microbial biomass

MBC and MBN concentrations were affected significantly ($P = 0.030$ and $P = 0.009$) by precipitation manipulation (Table 2) and this affected the MBC and MBN responses to warming. Warming increased the MBC and MBN concentrations by 3.3% and 22.3%, on average, in the precipitation control plots, and reduced MBC and MBN concentration by an average of 9.2% and 10.8% in the reduced precipitation plots (Table 3). However, in the elevated precipitation plots, warming lowered the MBC concentration by an average of 3.1%, but increased the MBN concentration by an average of 17.9% (Table 3). Soil microbial biomass concentrations varied among sampling times (all $P < 0.001$). Both MBC and MBN concentrations were affected significantly ($P = 0.005$ and $P = 0.049$) by the precipitation manipulation × time interaction, while the MBN concentration was affected significantly ($P = 0.049$) by the warming × time interaction (Table 2).

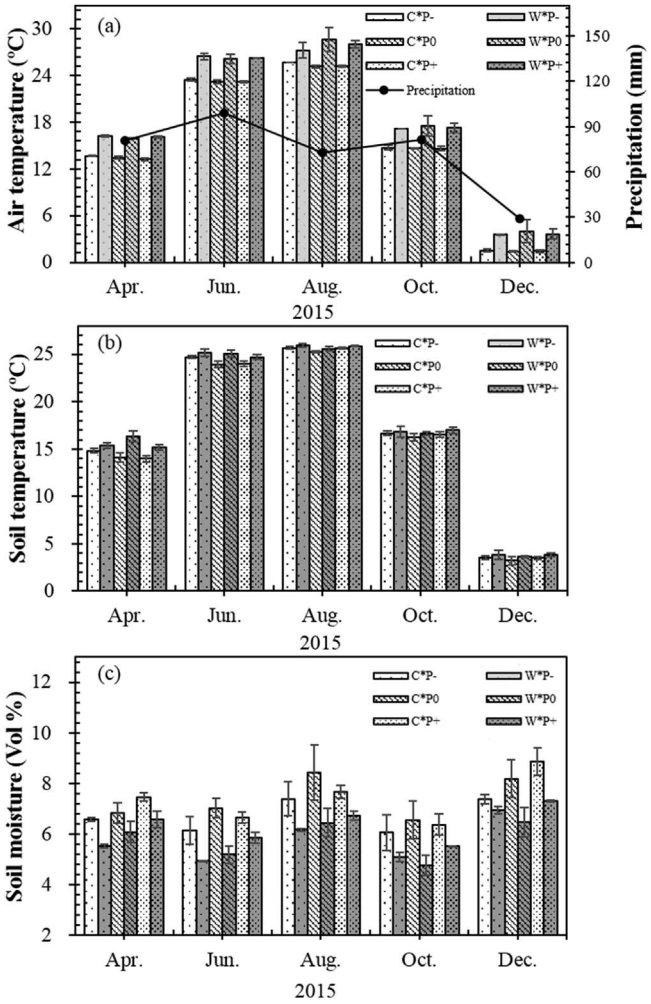


Fig. 1. Temporal variations in air temperature and precipitation (a), soil temperature (b), and soil moisture (c) among treatments (n = 3). Vertical bars indicate the standard error. C*P- = air temperature control and reduced precipitation; W*P- = air warming and reduced precipitation; C*P0 = air temperature control and precipitation control; W*P0 = air warming and precipitation control; C*P+ = air temperature control and elevated precipitation; and W*P+ = air warming and elevated precipitation.

Table 2
ANOVA results showing the responses of soil microbial biomass and soil extracellular enzyme activities to warming, precipitation manipulation, and their interaction.

Effect	df	MBC		MBN		MBC/N		BG		NAG		LAP		AP	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
P	12	4.79	*	7.19	**	4.78	*	0.41	ns	1.88	ns	6.58	*	0.39	ns
W	12	1.61	ns	2.86	ns	5.80	*	0.06	ns	0.66	ns	1.01	ns	1.58	ns
T	12	213.7	**	69.79	**	53.36	**	18.96	**	26.40	**	14.57	**	169.2	**
P*T	12	2.22	ns	3.53	ns	0.29	ns	0.47	ns	0.72	ns	1.63	ns	9.58	**
T*P	12	5.26	**	2.87	*	3.43	*	1.14	ns	7.54	**	2.38	ns	19.67	**
T*W	12	3.17	ns	3.30	*	4.82	*	0.76	ns	0.71	ns	1.31	ns	21.29	**
T*P*W	12	1.57	ns	2.14	ns	2.82	ns	0.46	ns	4.82	**	1.13	ns	22.18	**

W = warming; P = precipitation manipulation; T = time. MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; MBC/N = ratio of MBC to MBN; BG = β-1,4-glucosidase; NAG = *N*-acetyl-glucosaminidase; LAP = L-leucine aminopeptidase; and AP = acid phosphatase. ns = not significant at the level of $P > 0.05$; * = significant at the level of $P < 0.05$; and ** = significant at the level of $P < 0.01$.

3.3. Soil extracellular enzyme activity

During the study period, LAP activity was affected by precipitation manipulation ($P = 0.012$; Table 2). The LAP activity declined by 17.5% in the unwarmed plots with reduced precipitation, and by 46.4% in the unwarmed plots with elevated precipitation (Table 3). The AP was impacted significantly by the warming × precipitation interaction ($P = 0.003$; Table 2). Compared to the ambient treatment, the AP activity in plots with warming and precipitation manipulation varied by −20.7% to 11.0% (Table 3). Enzyme activities of BG, NAG, LAP, and AP varied among sampling times (all $P < 0.001$). The NAG and AP activities were affected significantly by the warming x precipitation x time effect (all $P < 0.001$; Table 2). Contrary to expectations, warming lowered the activity of the BG, NAG, LAP, and AP in precipitation control plots, but increased their activity in elevated precipitation plots (Table 3). The RDA analysis illustrated the temporal variability in extracellular enzyme activities, as RDA1 (37.3% of variance) and RDA2 (10.5% of variance) distinguished samples collected at five sampling times (Fig. 2). Together, soil temperature, soil moisture, and the inorganic N concentration explained 44.0% of variation in the extracellular enzyme activities (Fig. 2).

3.4. Soil microbial community composition and structure

Bacterial community diversity was not affected by the experimental treatments, but fungal community diversity was significantly affected by precipitation manipulation ($P = 0.011$) and the warming × precipitation interaction ($P = 0.017$; Table 4). Fungal community diversity decreased by 11.1% and 52.6% when precipitation was reduced in unwarmed plots and warmed plots, respectively, while there was 24.4% less fungal community diversity in the unwarmed plots with elevated precipitation and 8.3% less fungal community diversity in warmed plots with elevated precipitation. Compared with the ambient treatment, a change in the temperature and precipitation regime were detrimental to fungal community diversity ($P = 0.009$; Table 3).

At the experimental site, bacterial communities were dominated by phylum Proteobacteria, followed by phylum Acidobacteria, while the fungal communities were dominated by phylum Ascomycota, followed by phylum Basidiomycota. The relative abundance of dominant bacterial and fungal phyla was not affected by warming or precipitation manipulation, although significant changes in the Proteobacteria ($P = 0.030$) and Acidobacteria ($P = 0.015$) occurred due to the warming × precipitation interaction (Fig. 3). Other effects on of temperature and precipitation regimes on minor bacterial and fungal phyla

Table 3
Mean soil microbial and enzymatic properties under different treatments during the study period, presented as mean ± standard error.

		C*P-	W*P-	C*P0	W*P0	C*P+	W*P+
Soil microbial biomass							
MBC (×10 mg C kg^{-1} soil)		48.3 ± 5.6ab	43.9 ± 7.3b	48.4 ± 6.7ab	50.0 ± 7.0ab	51.2 ± 8.8a	49.6 ± 7.9ab
MBN (mg N kg^{-1} soil)		15.8 ± 2.6ab	14.1 ± 1.9ab	16.5 ± 2.2ab	20.2 ± 2.8a	13.5 ± 2.0b	16.0 ± 2.0ab
MBC/N		41.5 ± 16.9ab	38.1 ± 12.6b	37.6 ± 13.1ab	30.2 ± 8.9ab	44.2 ± 12.6a	39.4 ± 14.5ab
Soil extracellular enzyme activity							
BG (nmol h^{-1} g^{-1} soil)		18.5 ± 1.8	17.5 ± 0.7	18.7 ± 1.7	17.1 ± 2.0	18.5 ± 1.5	20.1 ± 1.2
NAG (nmol h^{-1} g^{-1} soil)		18.0 ± 5.3	17.8 ± 5.2	18.3 ± 3.8	13.3 ± 3.2	20.3 ± 4.6	20.7 ± 3.6
LAP (nmol h^{-1} g^{-1} soil)		25.3 ± 5.0ab	24.8 ± 4.1ab	30.6 ± 3.8a	29.9 ± 3.3a	16.4 ± 1.2b	24.3 ± 1.8ab
AP (nmol h^{-1} g^{-1} soil)		4.2 ± 1.2ab	4.6 ± 1.4ab	4.8 ± 1.2ab	3.8 ± 1.0b	3.8 ± 0.8b	5.3 ± 1.0a
Soil microbial community							
Chao 1	Bacteria (×10^3)	4.2 ± 0.6	4.4 ± 0.3	4.5 ± 0.2	3.8 ± 0.2	4.4 ± 0.1	4.1 ± 0.4
	Fungi (×10^2)	2.7 ± 0.1	1.7 ± 0.2	2.3 ± 0.0	2.6 ± 0.5	2.2 ± 0.2	2.1 ± 0.3
	Bacteria/Fungi	15.3 ± 2.3	25.8 ± 1.9	19.2 ± 20.9	16.1 ± 2.9	20.4 ± 2.2	20.4 ± 2.9
Shannon	Bacteria	9.5 ± 0.2	9.7 ± 0.1	9.8 ± 0.1	9.5 ± 0.1	9.7 ± 0.0	9.4 ± 0.1
	Fungi	2.6 ± 0.1a	1.4 ± 0.1b	3.0 ± 0.2a	3.0 ± 0.4a	2.2 ± 0.3a	2.7 ± 0.3a
	Bacteria/Fungi	3.6 ± 0.1b	6.9 ± 0.4a	3.4 ± 0.2a	3.3 ± 0.4a	4.5 ± 0.6b	3.6 ± 0.4b
Observed species	Bacteria (×10^3)	2.2 ± 0.2	2.3 ± 0.1	2.4 ± 0.1	2.1 ± 0.1	2.3 ± 0.0	2.2 ± 0.1
	Fungi (×10^2)	1.9 ± 0.1	1.2 ± 0.3	1.8 ± 0.1	1.9 ± 0.4	1.5 ± 0.2	1.5 ± 0.2
	Bacteria/Fungi	11.6 ± 1.4	21.2 ± 3.3	13.7 ± 0.8	12.0 ± 2.6	16.0 ± 2.1	14.9 ± 1.4
Phylogenetic diversity	Bacteria (×10^2)	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	1.3 ± 0.1
	Fungi (×10^2)	2.7 ± 0.1	1.7 ± 0.2	2.3 ± 0.0	2.6 ± 0.5	2.2 ± 0.2	2.1 ± 0.3
	Bacteria/Fungi	0.5 ± 0.0b	0.9 ± 0.1a	0.6 ± 0.0ab	0.5 ± 0.1b	0.7 ± 0.1ab	0.7 ± 0.1ab

W = warming; P = precipitation manipulation; C*P- = air temperature control and reduced precipitation; W*P- = air warming and reduced precipitation; C*P0 = air temperature control and precipitation control; W*P0 = air warming and precipitation control; C*P+ = air temperature control and elevated precipitation; and W*P+ = air warming and elevated precipitation. MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; MBC/N = ratio of MBC to MBN; BG = β-1,4-glucosidase; NAG = *N*-acetyl-glucosaminidase; LAP = L-leucine aminopeptidase; and AP = acid phosphatase. Different lower-case letters represent significant differences ($P < 0.05$).

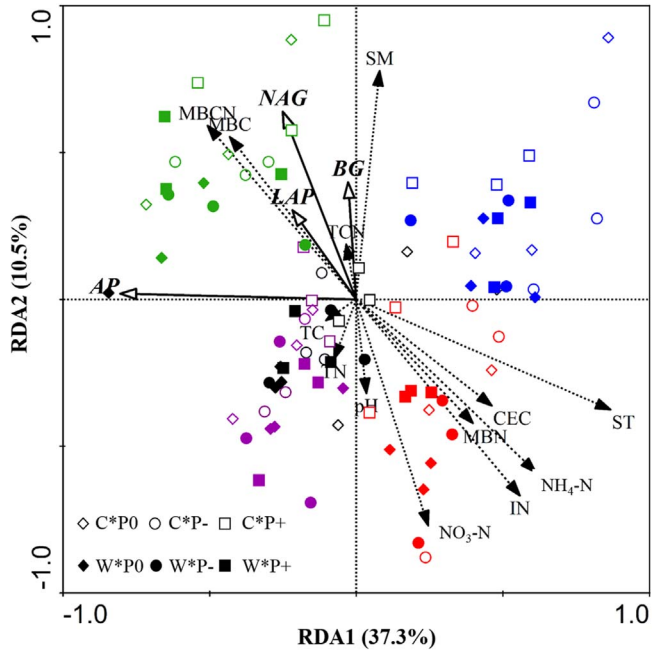


Fig. 2. Result of redundancy analysis (RDA) based on soil extracellular enzyme activities and soil properties. ST = soil temperature; SM = soil moisture; CEC = cation exchange capacity; TC = total C; TN = total N; TCN = ratio of TC to TN; and IN = inorganic N. Samples in the same colors indicate sampling in the same time (purple: April; red: June; blue: August; black: October; green: December). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

are shown in Fig. 3. Compared with the ambient treatment, the relative abundance of Acidobacteria was altered significantly ($P = 0.029$) by −1.4% to 9.3% due to the treatments, while the relative abundance of Proteobacteria varied by −8.9% to 0.2%, which was not significant. It is notable that the relative abundance of Proteobacteria decreased with a concomitant increase in the abundance of Acidobacteria (Fig. 3). The RDA revealed that warming and precipitation manipulations were associated with distinctive bacterial and fungal groups, and the RDA explained 31.5% and 50.2% of the variation in bacterial and fungal communities, respectively. Together, the variables of soil CEC, soil moisture, the inorganic N concentration, and TC:TN ratio explained 50.0% of variation in the bacterial community, while soil temperature, soil moisture, pH, and the TN concentration explained a 40.0% of variation in the fungal community (Fig. 4).

4. Discussion

4.1. Soil microbial biomass and extracellular enzymatic responses to climate change drivers

Our findings partially supported the first hypothesis, as warming alone had a minimal stimulating effect on soil microbial biomass and extracellular enzyme activities in precipitation control plots and elevated precipitation plots, but had a passive effect in reduced precipitation plots (Table 3). Although the effects of warming on soil microbial biomass and extracellular enzyme activity were not remarkable, there was a significant interaction effect of warming and precipitation, and/or time (Table 2). These results were contradictory with many studies that showed that warming increased the soil microbial biomass and extracellular enzyme activities (Rui et al., 2011; Zhou et al., 2013; Zhao et al., 2016), and suggested that warming effects were environmentally dependent. Other climate change studies have revealed that temporal shifts in natural precipitation, soil microclimate, and plant root exudates could regulate the composition and function of soil microbial communities, as well as soil extracellular enzyme activities (Puissant et al., 2015; Smith et al., 2015). Similarly, responses of soil physicochemical properties and soil microbes to treatments showed temporal variation in our study (Tables 1 and 2). Thus, the warming condition effect could be due to the inter-annual differences in natural precipitation and the temporal changes of the available soil nutrients.

Similar to the first hypothesis, the second hypothesis was also partially supported, as reduced precipitation decreased the soil microbial biomass and extracellular enzyme activities, but elevated precipitation only increased MBC and LAP (Table 3). These results may be due to the shifts in soil moisture due to precipitation manipulation and this is consistent with reports that soil moisture could affect the soil microbial community and extracellular enzyme activities by regulating the diffusion of available nutrients (Geng et al., 2014). Meanwhile, the dissimilar responses of soil extracellular enzymes to elevated precipitation may suggest that the biological factors involved in the production of soil extracellular enzyme and the physicochemical controls on substrate diffusion and hydrolysis reactions vary among the enzymes (Machmuller et al., 2016). On the other hand, precipitation manipulation appeared to affect the effect of warming on soil microbial biomass and extracellular enzyme activity. The warming effect could also be attributed to soil moisture limitation induced by the drying effect of warming. Although warming increased the soil temperature, the concomitant soil drying could also limit the diffusion of soluble nutrients and the mobility of soil microbes, which in turn could decrease the accumulation of soil microbial biomass (Steinweg et al., 2013; Geng et al., 2014). Soil moisture at the study site was naturally low due to the loamy sand soil and unusual low precipitation in 2015 (only 54% of the 30-year average; Table S2).Warming may reduce the soil microbial biomass and extracellular enzyme activities by exacerbating drought conditions at the study site. Since enzyme activity data in the present study represent potential activities, our findings might not fully reflect enzymatic responses to soil moisture limitation in the field. However, our findings were in line with those of other studies on warming, which showed that warming might suppress the soil microbial biomass and extracellular enzyme activity through decreasing moisture availability in water-limited environments (Steinauer et al., 2015). In combination, these results confirm the third hypothesis about the soil microbial biomass and extracellular enzyme activity, and suggested the magnitude of change in microbial biomass and functions in warmer soils is

Table 4
ANOVA results showing the responses of soil microbial community properties to warming, precipitation manipulation, and their interaction.

	df	Chao 1						Shannon						Observed species						Phylogenetic diversity					
		B		F		B/F		B		F		B/F		B		F		B/F		B		F		B/F	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
P	12	0.07	ns	0.63	ns	1.02	ns	0.44	ns	6.71	*	12.02	**	0.00	ns	1.48	ns	1.52	ns	0.09	ns	0.63	ns	0.70	ns
W	12	0.76	ns	1.84	ns	1.73	ns	1.97	ns	1.28	ns	5.66	*	1.00	ns	1.01	ns	1.71	ns	1.86	ns	1.84	ns	2.25	ns
P*W	12	0.83	ns	2.88	ns	4.87	*	2.48	ns	5.84	*	15.50	**	2.02	ns	2.24	ns	4.55	*	3.05	ns	2.88	ns	5.71	*

W = warming; P = precipitation manipulation; B = bacteria; F = fungi; and B/F = ratio of bacteria to fungi. ns = not significant at the level of $P > 0.05$; * = significant at the level of $P < 0.05$; and ** = significant at the level of $P < 0.01$.

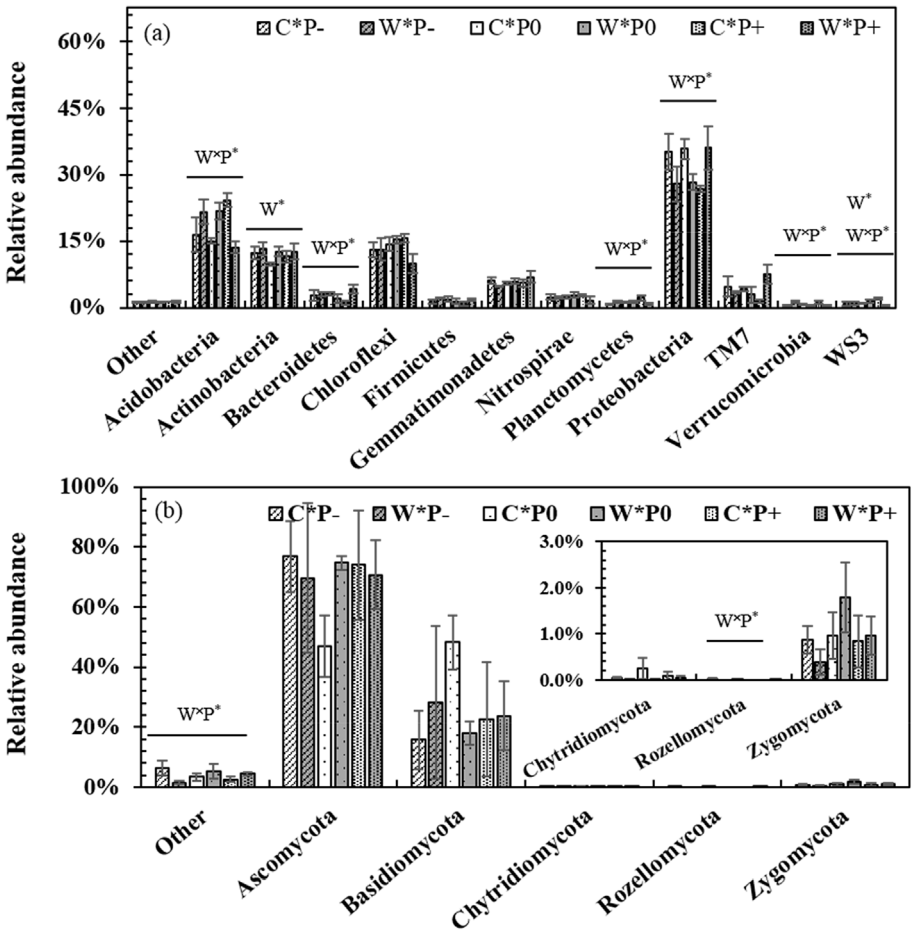


Fig. 3. Relative abundance of soil bacterial phyla (only relative abundance > 1% are presented) (a) and fungal phyla (b) in each treatment plot. Vertical bars indicate the standard error. W = Warming; P = Precipitation; C*P- = air temperature control and reduced precipitation; W*P- = air warming and reduced precipitation; C*P0 = air temperature control and precipitation control; W*P0 = air warming and precipitation control; C*P+ = air temperature control and elevated precipitation; and W*P+ = air warming and elevated precipitation. * = significant at the level of $P < 0.05$.

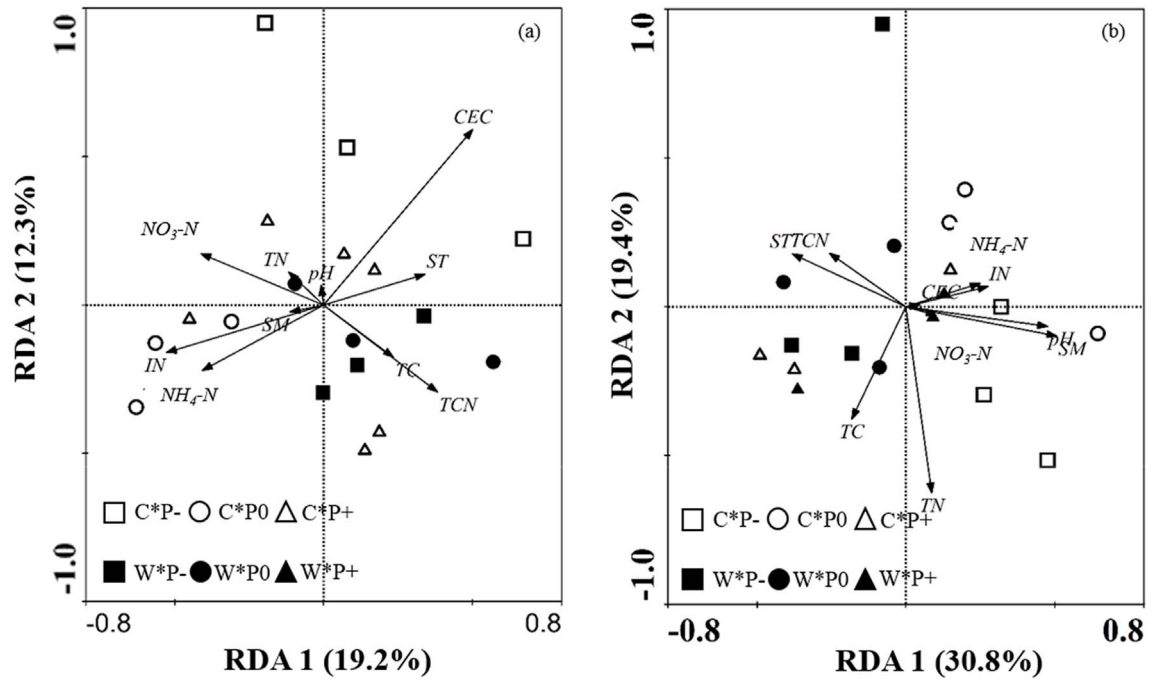


Fig. 4. Redundancy analysis (RDA) results of bacterial (a) and fungal (b) community. C*P- = air temperature control and reduced precipitation; W*P- = air warming and reduced precipitation; C*P0 = air temperature control and precipitation control; W*P0 = air warming and precipitation control; C*P+ = air temperature control and elevated precipitation; and W*P+ = air warming and elevated precipitation. ST = soil temperature; SM = soil moisture; CEC = cation exchange capacity; TC = total C; TN = total N; TCN = ratio of TC to TN; and IN = inorganic N.

dependent on soil moisture.

4.2. Soil microbial community responses to climate change drivers

In support of the fourth hypothesis, we found that warming had a differential effect on both bacterial and fungal community structures under each precipitation regime (Fig. 4). Meanwhile, the warming effect on the bacterial and fungal communities (i.e., richness and diversity) varied among precipitation treatments (Table 3). Together, these findings indicate that the warming effect on the growth and structure of a soil microbial community may be controlled by precipitation manipulation, and the alterations in the microbial community structure were affected by treatment-induced shifts in soil moisture. Certain microbial groups may be more resistant to changes in soil moisture than others. For example, some groups have special adaptive traits to desiccation, such as thickened cell walls, resulting in greater tolerance to fluctuations in soil moisture (Zhang et al., 2016a). As the redox state of soil is anaerobic and that moisture controls nutrient diffusion, soil moisture could be a critical factor affecting microbial community structure via interacting moisture-dependent processes (Steinweg et al., 2013; Geng et al., 2014).

Contrary to our hypothesis, only fungal community diversity significantly responded to precipitation manipulation. Bacteria communities have small-scale niches in soil and are less often symbiotic with plants, compared to fungal communities (Sun et al., 2017a); thus bacterial community diversity may show less response to the shorts in soil conditions that were directly and indirectly induced by treatments. Although interception of rainwater by the seedling canopy and the abnormally low precipitation in 2015 (only 54% of the 30-year average; Table S2) may have exacerbated the effect of precipitation manipulation on soil moisture (Park et al., 2016; Yun et al., 2016), precipitation manipulation may indirectly affect the fungal community diversity by altering other soil properties (e.g., pH, TC, and TN). Our experimental treatments also affected soil pH, TC, TN, and inorganic N during the study period (Table 1), explaining a combined 34% of the variation in the fungal community. Particularly, the general decrease in soil pH occurred under the elevated precipitation regime, which might have been caused by additional input of acid deposition through irrigated rainwater containing diverse acidic ions (Kim et al., 2010). An acidic soil condition under elevated precipitation may act as environmental stress for fungal communities, and consequently alter soil fungal diversity.

The major alterations in bacterial communities were observed in specific dominant phyla (e.g., Proteobacteria and Acidobacteria), which occupy unique niches (Fig. 3). Some climate change studies suggested that climate change drivers could change the structure and composition of soil microbial communities by selecting species that can thrive in the new environments (Zhang et al., 2016a). Considering the lack of plant growth responses to the treatments during the study period (Park et al., 2016), our results also indicate that direct effects on soil may contribute to the changes in bacterial and fungal communities rather than indirect effects caused by the feedbacks due to changes in plant growth (Fig. 4). Because actual climate change can be more gradual than the changes observed in short-term experiments, our results might not reflect the long-term changes in the soil microbial communities. As such, further monitoring is required to confirm whether the detected pattern will persist under more gradual climate changes over a longer time scale.

4.3. Key factors related to the soil microbial and extracellular enzymatic responses to climate change drivers

Previous studies reported that soil microbes and enzymes are generally influenced by the soil microclimate (temperature and moisture; Zhou et al., 2013). In this study, soil moisture was the primary limiting factor and more important than temperature in terms of soil microbial and extracellular enzymatic responses to climate change drivers. Besides moisture, soil microbial and extracellular enzymatic responses to climate change drivers were also related to other physicochemical properties (e.g., pH, CEC, TN, and inorganic N; Figs. 2 and 4). Studies by Fierer et al. (2007) and Sun et al. (2017b) showed that soil pH was closely related to the changes in soil microbes and extracellular enzyme activities across biomes. In addition, previous studies have suggested that soil inorganic N is also a key factor in structuring the microbial community, and that P- and C-acquisition enzymes are highly related to inorganic N, indicating the importance of inorganic N for microbial responses to climate change drivers (Zhou et al., 2013; Zhang et al., 2016b). Furthermore, competition for nutrients among plants and free-living soil microbes could make nutrient availability a limiting factor for soil microbes (Zhang et al., 2005).

These results also provide some insights into the relationship between function and structure of soil microbial communities, as indicated by the relationships between the microbial parameters and extracellular enzyme activities (Table S4 and Fig. 4). The high correlations of microbial properties (i.e., biomass, richness, and diversity) with enzyme activities indicate that shifts in microbial properties were associated with changes in enzyme activities. Given that different microbial groups target different substrates and produce different enzymes, the shifts in soil microbial community composition caused by climate change drivers may indirectly influence soil enzyme activities.

4.4. Implications of alterations to soil microbial communities and extracellular enzymes induced by climate change

We observed that fungal community diversity and composition of the microbial community (e.g., ratio of bacterial to fungal communities) were significantly responsive to precipitation manipulation or the interaction of warming and precipitation manipulation (Table 4). The alteration in composition of the microbial community may further alter the decomposition process from bacterial-to fungal-based channels (Zhang et al., 2005). Fungi have greater C assimilation efficiency, lower N demand, and more polymers (e.g., melanin and chitin) in their cell walls than bacteria. Hence, shifts in the ratio of the fungal community to the total soil microbial community may further influence the transformation of organic C, a pool of SOM, and available N in soil (Bailey et al., 2002).

An earlier study at the same site found that the increasing effect of warming on soil respiration varied among different precipitation manipulations (Li et al., 2017b). In the present study, warming effects on soil microbes and extracellular enzymes also varied across precipitation regimes (Table 3 and Fig. 1). Given that the quality and quantity of substrates may be the main factors regulating soil respiration (Zhang et al., 2005), the similar results, the results suggest that the soil respiration response to climate change drivers might have been due to an alteration in the degradation of recalcitrant SOM by soil extracellular enzymes, as well as changes in the soil microbial community structure. These results indicate that in water-limited environments, soil moisture may play greater roles in providing positive or negative feedback by regulating the decomposition of SOM and CO_2 released from soil.

In addition, climate change drivers may affect soil N transformations. Similar changes occurred in the inorganic N concentration and microbial community structure, in response to climate change drivers. Furthermore, it was found that the inorganic N concentration was highly related to N-acquisition enzyme activities as well as the composition and structure of the soil microbial communities (Table 4, Figs. 2 and 4). These results implied that bioavailable N concentration in soil contributed to structure the microbial community, consistent with the observation that climate change drivers stimulated the diversity and complexity of the microbial communities, favoring microbes capable of acquiring inorganic N (Zhang et al., 2016b).

In conclusion, this study revealed the direct effects of multiple climate change drivers (warming and precipitation manipulation) on soil microbial communities. Soil moisture may induce direct effects of

climate change drivers in water-limited environments, especially on the fungal community in soils. Indirect effects of plants were minimal because of the lack of physiological and growth responses of *P. densiflora* seedlings to climate change drivers during the study period (Park et al., 2016; Yun et al., 2016). These results indicate that soil microbial biomass and community structure, as well as extracellular enzyme activity, may respond to climate change drivers due to direct changes in soil environment rather than indirect feedback processes, at least in the short-term. The study findings need to be confirmed in older forest plantations, since 2-year-old seedling cannot represent mature *P. densiflora* forests, which dominate South Korea's forest cover (Kim et al., 2016). Future studies should include more replicate field plots because limited replication in the present study hindered our interpretation of the effect of climate change drivers. Nevertheless, our findings can help to understand the shifts in soil microbes and associated ecosystem functioning in *P. densiflora* plantations undergoing climate change in South Korea.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2018.02.014.

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